Electronic Supplementary Material (ESI) for New Journal of Chemistry.
This journal is © The Royal Society of Chemistry and the Centre National de la Recherche Scientifique 2019

Electronic Supplementary Information

A new amplification strategy for quartz crystal microbalance miRNA sensor based on selective interactions between Metal-organic framework and miRNA

Ding Ma,[a] Sheng-Run Zheng,*[a] Jun Fan,[a] Song-Liang Cai,[a] Zong Dai,[b] Xiao-Yong Zou,[b] Shao-Hua Teng[c] and Wei-Guang Zhang,*[a]

[a.] *School of Chemistry, South China Normal University, Guangzhou, 510006, China.*

[b.] *School of Chemistry, Sun Yat-Sen University, Guangzhou 510275, China.*

[c.] *School of Computer Science and Technology, Guangdong University of Technology, Guangzhou 510006, China.*

**To whom correspondence should be addressed.*

E-mail: zhengsr@scnu.edu.cn; wgzhang@scnu.edu.cn

Fax: +86-20-39310210

Tab S1. miRNA Sequence

Name	Abbreviation	Sequence
Surface capture probe	SH-PRNA	5'-SH-AACUAUACAACCUACUACCUCA-3'
Capture probe	PRNA	5'-AACUAUACAACCUACUACCUCA-3'
Target miRNA	let-7a	5'-UGAGGUAGUAGGUUGUAUAGUU-3'
SH-target	SH-let-7a	5'-SH-UGAGGUAGUAGGUUGUAUAGUU-3'
Single base mutation target	let-7c	5'-UGAGGUAGUAGGUUGUAUGGUU-3'
Single base mutation target	let-7e	5'-UGAGGUAGGAGGUUGUAUAGUU-3'
Double base mutation target	let-7b	5'-UGAGGUAGUAGGUUGUGUGGUU-3'
FL-probe	FAM-PRNA	5'-FAM-AACUAUACAACCUACUACCUCA-3'
FL-target	FAM-let-7a	5'-FAM-UGAGGUAGUAGGUUGUAUAGUU-3'

Methods

Materials and Measurements. $ZrCl_4$ and 1,4-benzenedicarboxylic acid purchased from were phased from Beijing HWRK Chem Co., LTD., China. HPLC-purified miRNA, RNase inhibitor, and DEPC-treated water were obtained from Sangon Co. Ltd. (Shanghai, China; DEPC =diethylpyrocarbonate). Vent (exo^-) DNA polymerase and the nicking endonuclease Nt.BstNBI were purchased from New England Biolabs. SYBR Green I (20 × stock solution in dimethyl sulfoxide, 20 μg mL^{-1}) was purchased from Genetimes Technology, Inc (Shanghai, China). All other reagents were of analytical grade and were commercially available from Guangzhou Chemical Reagent Factory and were used without further purification. X-ray powder diffraction measurements were measured by using an Ultima IV X-ray powder diffractometer (Kurary, Tokyo, Japan) at 40 kV, 40 mA equipped with Cu Kα radiation (k = 1.5406 Å). Zeta potentials were measured by the Zetasizer Nano ZS90 from Malvern Instruments with the liquid concentration of 0.75 mg/mL. ECD spectra were recorded using a JASCO J815 Research-Grade Circular Dichroism (CD) Spectrometer. Fluorescence spectra were recorded using a Hitachi F-4600 spectrofluorometer. The EXPAR and the real-time fluorescence measurements were performed with a 7500 Real-Time PCR System (Applied Biosystems, USA).

Synthesis and particle size screening of UiO-66 with different zeta potentials. Three kinds of UiO-66 with different zeta potentials was synthesized according to literature, and denote as UiO-66-z1、UiO-66-z2 and UiO-66-z3.

Preparation of UiO-66-z1. The UiO-66-z1 was synthesized according to the reported approaches with modifications, a mixture of $ZrCl_4$ (1.087g) and 1,4-benzenedicarboxylic acid (BDC, 0.525g) was dissolved in N, N- dimethylformamide (DMF, 15 mL) respectively at room temperature. Then the solutions were mixed, stirred for 20 min, and heated at 125 ºC for 24 h. The solid products were collected by centrifugation and washed three times with DMF and three times with MeOH to remove the residual reactant. Then the product was exchanged by fresh methanol for two days. The result product was dried at 60 ºC and then activated at 120 ºC for 24 h.

Preparation of UiO-66-z2. $ZrCl_4$ (0.2174g), DMF (3 mL), and triocylamine (0.12mL) was mixed and stirred until dissolved, the solution was aging for 48h. Mixture of BDC (0.1050g) in DMF (3 mL) and PVP (0.1415g) in 15ml DMF lead to another solution. Then, two solution was further mixed and transferred to the autoclave and heated at 125 ºC for 24 hours. After cooling, the solid products were collected and dried as same as to that in UiO-66-z1.

Preparation of UiO-66-z3. BDC (0.105g), DMF (3 mL), and triocylamine (0.12mL) was mixed and stirred until dissolved, the solution was aging for 48h. Mixture of $ZrCl_4$ (0.2174g) in 3mL DMF and PVP (0.1415g) in 15ml DMF lead to another solution. Then, two solution was further mixed and transferred to the autoclave and heated at 125 ºC for 24 hours. After cooling, the solid products were collected and dried as same as to that in UiO-66-z1.

particle size screening of UiO-66-z1. The UiO-66-z1 (10 mg) is dispersed in methanol (20 mL) and the suspension is formed by ultrasonic for 1 hours. Then, the suspension is centrifuged in different rotating speed for several minutes, and the upper suspension is taken to obtain UiO-66-z1 with specific particle size. UiO-66-z1 with particle size of 200, 300, 600 and 900 nm can be obtained by controlling the rotating speed.

Assembly of QCM-miRNA biosensors. The QCM-miRNA biosensors were prepared by using a two-step assembling process. first, the quartz gold piece was soaked in piranha solution (98% H_2SO_4:30% H_2O_2 (v/v) = 7:3) for 10 minutes, rinsed with deionized water, and dried with nitrogen. Secondly, the pretreated quartz gold piece was immersed in the mixture of 6-mercapto-1-hexanol (0.025 mM) and SH-PRNA (50 nM) solution for 24 hours at 4 °C to form a miRNA-based biochip.

The preparation of Let-7a@UiO-66. 2mg UiO-66 powder was dispersed in 2mL PBS buffer (pH=7.4) to form a UiO-66 suspension though ultrasonic for 30min (solution A). And the initial Let-

7a solution (solution B) was prepared by dispersing Let-7a with DEPC water and oscillate evenly to a concentration of 20 μM. Then, take certain volumes of solution A and B by using a liquid shift gun, then add PBS buffer until the final volume is 2ml, supersonic in ice water bath to make it completely mixed.

On line detection by QCM. All on-line detections were implemented on a home-made aqueous media measuring QCM system (Fig. S1). The system consisted of a QCM biosensor, a reaction cell, an oscillator detector, a frequency counter and a computer. The AT-cut quartz crystals (13.0 mm diameter, 5MHz resonant frequency) were covered with gold electrodes on both sides. To prevent MOFs deposition, QCM sensors test face down. For QCM detection, firstly, phosphate buffer was injected into the QCM cell, and the crystal frequency was measured as a background signal. After the signal change was less than 1 Hz of frequency drift in 5 min, the experiment could be continued. Then, the PBS solution/suspension was injected into the QCM liquid cell until it was filled, and the solution/suspension in the QCM cell was kept still until a stable signal was obtained.

Detection of interaction between UiO-66 and miRNA by QCM. The thiol-modified miRNAs were bonded to the surface of quartz crystal gold, then assembled on QCM sample cell, and the certain concentration of TRNA@UiO-66 suspension was injected into the sample pool to record the frequency change data.

Detection of interaction between UiO-66 and miRNA by ECD. The preparation of ECD test samples are based on our experimental conditions: 40μM PRNA/TRNA solution and UiO-66 solution with QCM experimental concentration was bathed in ice water and dispersed by sonication for 1h, then stored in 4℃ overnight. In CD spectroscopy measurements, slit width was set to 1.0 nm, the spectra were collected from 220nm to 340nm. Test the solvent spectrum as the background peak.

Detection of interaction between UiO-66 and miRNA by FL. The fluorescence measurements were performed with excitation slit width 10.0 nm and emission slit width 10.0 nm. The emission spectra were collected from 500 to 600 nm under the excitation wavelength of 480 nm, and the fluorescence intensity at 518 nm is used for analysis. Keeping the concentration of miRNA constant to 50 nM, and the preparation method is consistent with the above experiment.

EXPAR method. The reaction mixtures for the EXPAR were prepared separately on ice as part A and part B. Part A consisted of Nt.BstNBI buffer, the amplification template, dNTPs, RNase inhibitor, and the miRNA target; part B consisted of ThermoPol buffer, the nicking endonuclease Nt.BstNBI, Vent (exo^-) DNA polymerase, SYBR Green I, and DEPC-treated water. Parts A and B were mixed immediately before being placed in the Real-Time PCR System. The EXPAR was performed in a volume of 20 μL containing the amplification template(1 μm), dNTPs (250 μm), Nt.BstNBI (0.4 U μL^{-1}), Vent (exo^-) DNA polymerase (0.05 U μL^{-1}), RNase inhibitor (0.8 U μL^{-1}), SYBR Green I (0.4 μg μL^{-1}), 1 × ThermoPol buffer (20 mM Tris–HCl, pH 8.8, 10 mm KCl, 10 mm $(NH_4)_2SO_4$, 2 mm $MgSO_4$, 0.1% Triton X-100; Tris =2-amino-2-hydroxymethylpropane-1,3-diol), and 0.5×Nt.BstNBI buffer (25 mM Tris–HCl, pH 7.9, 50 mM NaCl, 5 mM $MgCl_2$, 0.5 mM dithiothreitol). The EXPAR was performed at 55°C, and the real-time fluorescence intensity was monitored at intervals of 30 s.

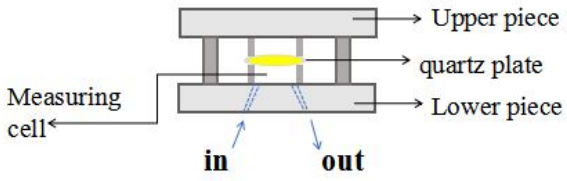

Fig. S1 Schematic diagram of Static detection quartz crystal microbalance sensor system.

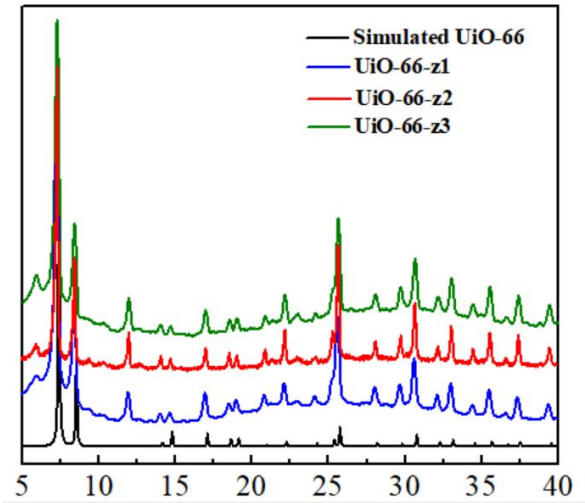

Fig. S2 The XRD of simulated and synthesized UiO-66.

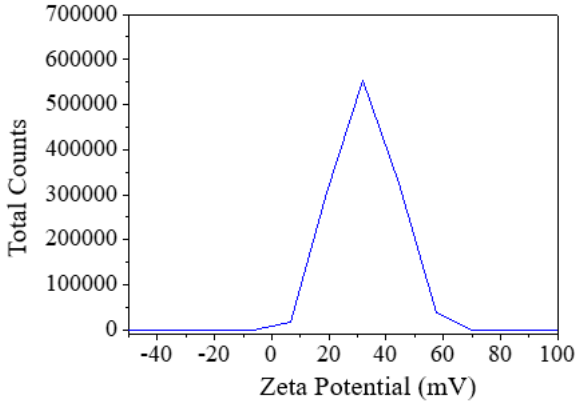

Fig. S3 The zeta potential of UiO-66-z1

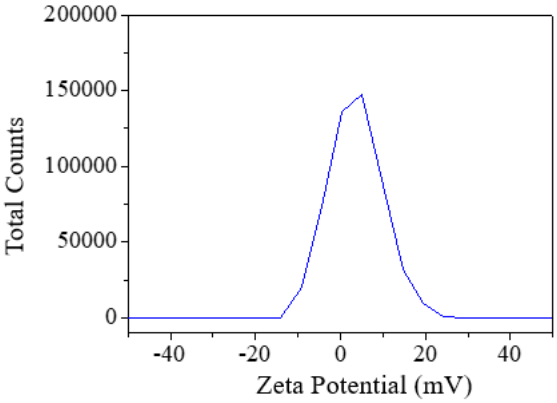

Fig. S4 The zeta potential of UiO-66-z2

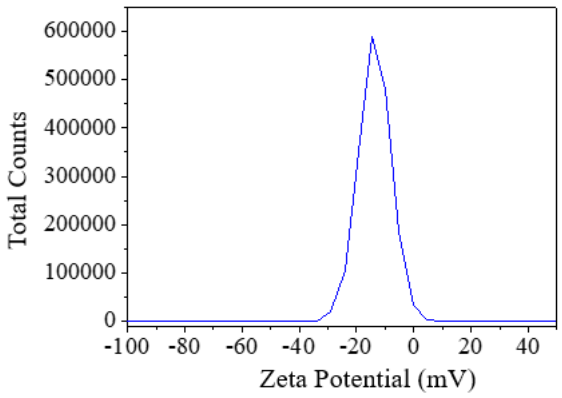

Fig. S5 The zeta potential of UiO-66-z3

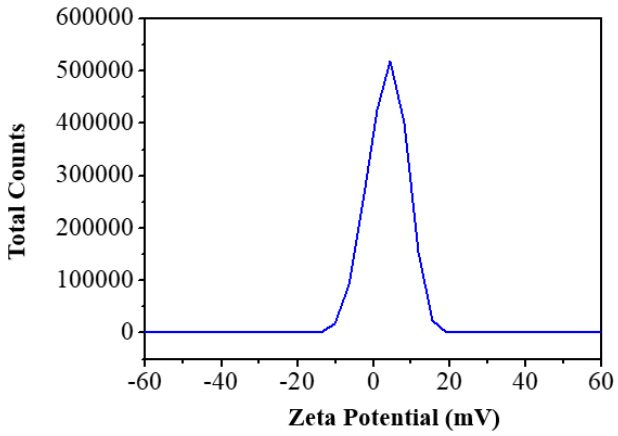

Fig. S6 The zeta potential of TRNA@UiO-66-z1.

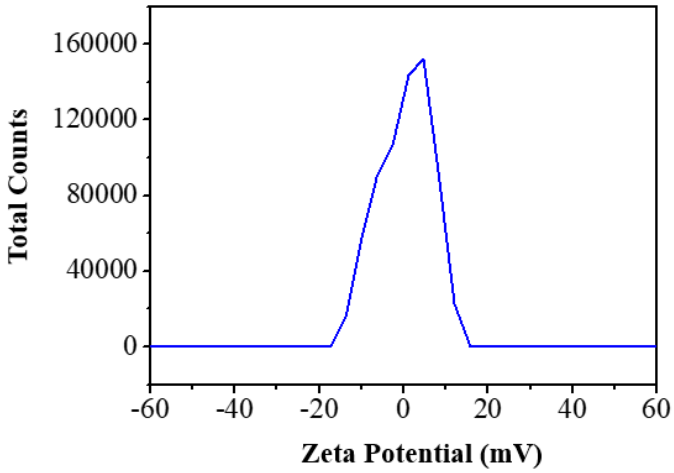

Fig. S7 The zeta potential of TRNA@UiO-66-z2.

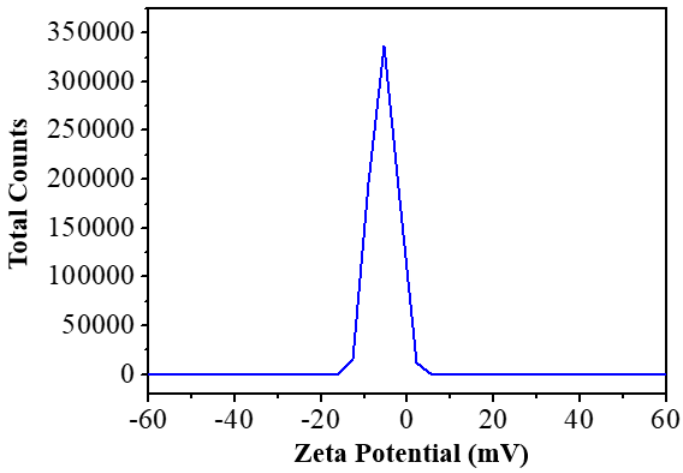

Fig. S8 The zeta potential of TRNA@UiO-66-z3.

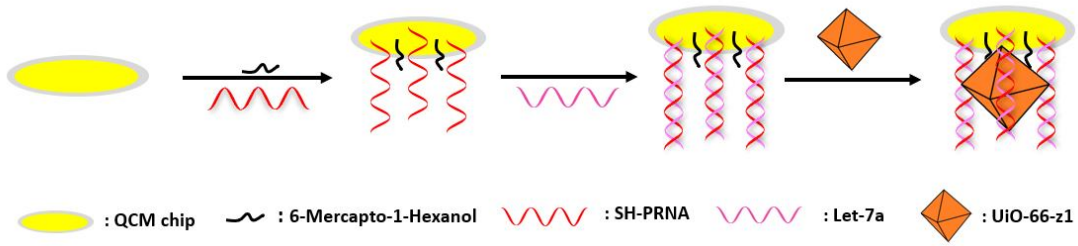

Fig. S9 Schematic diagram that shows the approach for study the interactions between dsmiRNA and UiO-66-z1 by QCM method.

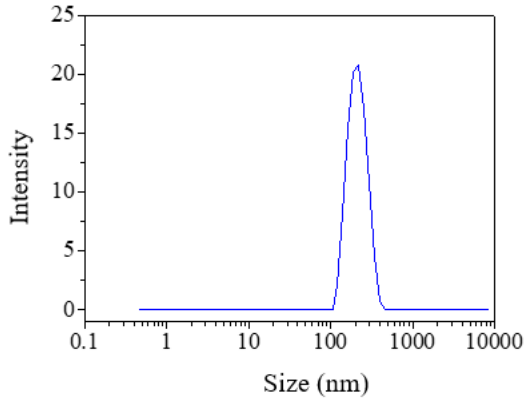

Fig. S10 The size distribution of UiO-66-z1 with average size of 200 nm

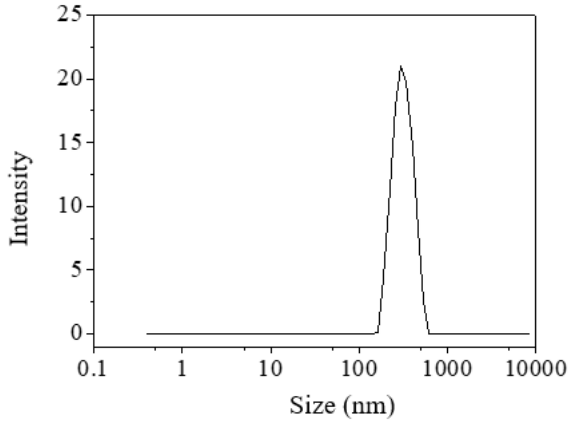

Fig. S11 The size distribution of UiO-66-z1 with average size of 300 nm

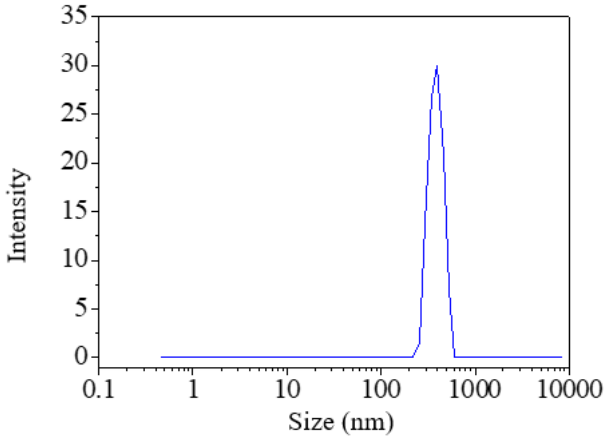

Fig. S12 The size distribution of UiO-66-z1 with average size of 600 nm

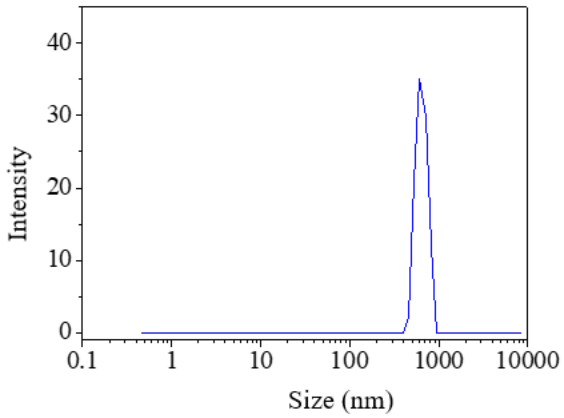

Fig. S13 The size distribution of UiO-66-z1 with average size of 900 nm

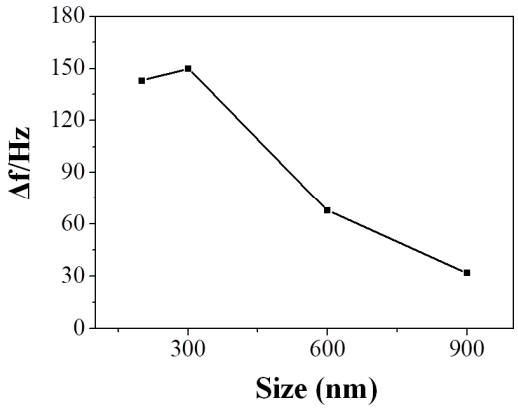

Fig. S14 The influence of size of UiO-66-z1 on frequency change.

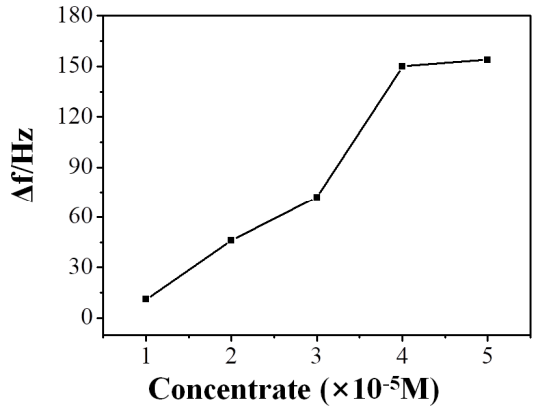

Fig. S15 The influence of concentration of UiO-66-z1 on frequency change.

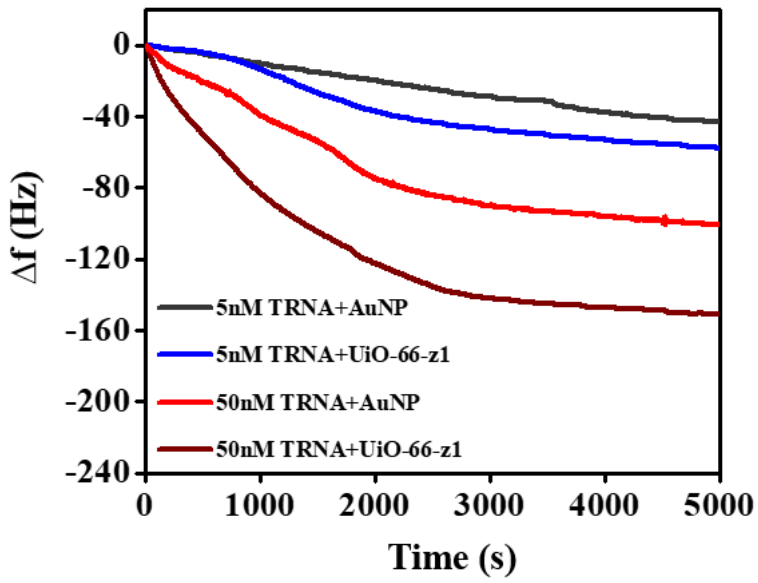

Fig. S16 Real-time frequency responses of the miRNA-modified QCM sensor with addition Let-7a with using UiO-66-z1 and Au nanoparticle as amplifier. The concentrations of the selected miRNAs are 5 and 50 nM.

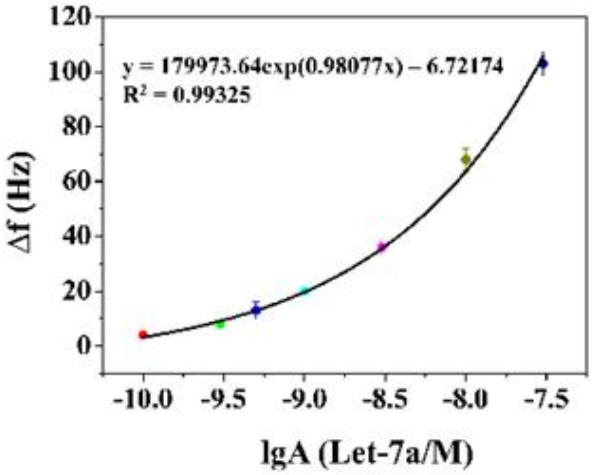

Fig. S17 The exponential relationship between frequency response and target Let-7a concentration. The error bars represent the standard deviation of three repeat measurements.

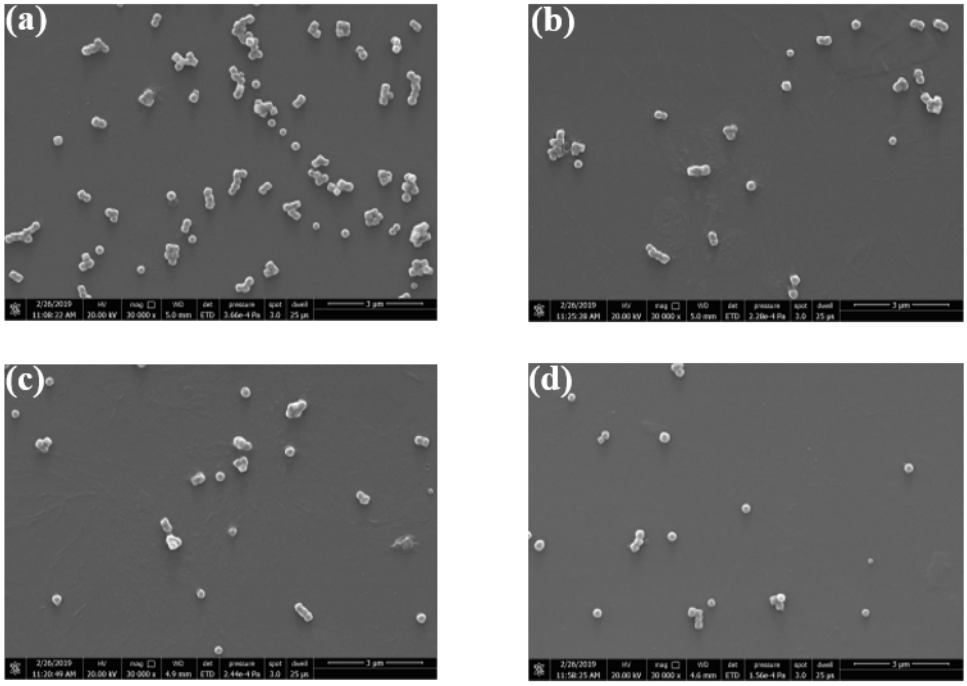

Fig. S18 The SEM of QCM sensor surface condition after UiO-66-z1 combine to let-7a of different concentration. From a to d: 50nM, 10nM, 5nM, 1nM.

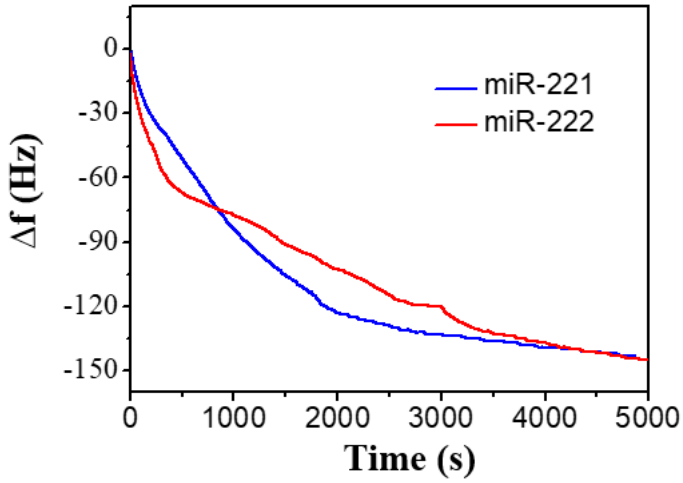

Fig. S19 Real-time frequency responses of the miRNA-modified QCM sensor with addition miR-221 and miR-222 with UiO-66-z1 as amplifier. The concentrations of the selected miRNAs are 50 nm.

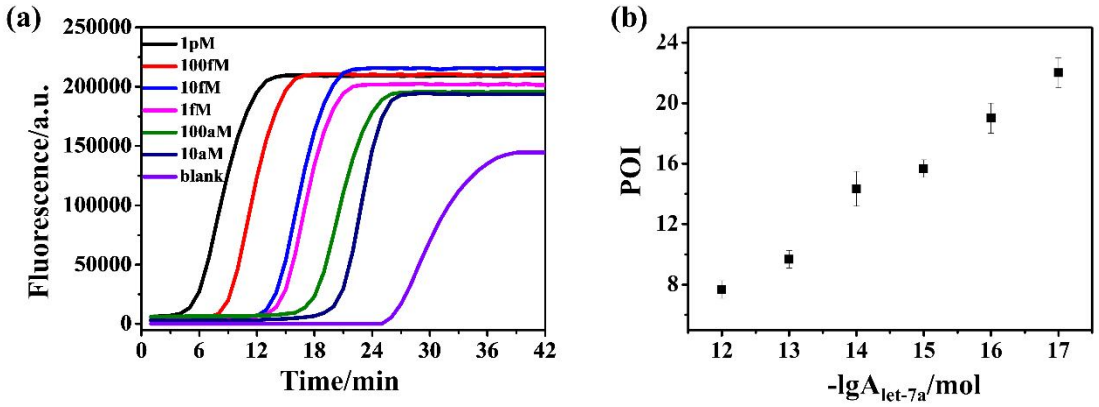

Fig. S20 (a) The real-time fluorescence curves produced by target M with different concentrations. From left to right, the target M concentrations are successively 1 pM, 100 fM, 10 fM, 1 fM, 100 aM, 10 aM and 0 (Blank); (b) the plots between POI values in the real-time fluorescence curves and logarithm (lg) of the target M concentrations. Error bars are estimated from the standard deviation of three replicate measurements at each data point.